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13. ABSTRACT *(Maximum 200 Words)*

The overall goal of this project is to understand the mechanism of regulation of human BRCA2 gene expression in order to explore the possibility of epigenetic malfunction in this mechanism, which may lead to sporadic breast cancer. BRCA2 mRNAs were only detected in dividing cells but not at all in quiescent cells. We have found a transcriptional silencer at the upstream of human BRCA2 gene. This silencer is active only in the quiescent cells but not in the dividing breast cells, thus explaining the absence of BRCA2 mRNA in the quiescent cells. The mechanisms of the activation and inactivation of this silencer in the quiescent and dividing cells, respectively, are presently unknown. We have shown that specific nuclear proteins from quiescent breast cell nuclear extract sequence-specifically binds to this silencer. We also have observed that at least some of the African-American breast cells may have alteration in this regulatory pathway. We hypothesize that the human BRCA2 gene is silenced in the quiescent stage of breast cells but is activated in the dividing cells by the inactivation of the silencer. Possible transient epigenetic malfunction in this silencer inactivation process by environmental factors in the dividing cells may lead to defect in DNA repair and subsequence onset of mutations in any key gene leading to oncogenesis. Our studies may relate this regulatory pathway with respect to the ethnic origin of the breast cells.

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Table of Contents

Introduction

The overall goal of this proposed project is to understand the mechanism of regulation of human BRCA2 gene expression in order to explore the possibility of epigenetic malfunction in this mechanism, which may lead to sporadic breast cancer. The majority (>95%) of human breast cancer happen sporadically and caused by mutations in a variety of genes (1-5). On the other hand, the familial breast cancers are caused by the defects in either of the two DNA repair protein genes, BRCA1 and BRCA2 (1). Possibility of epigenetic malfunction in the expression of these genes in developing sporadic breast cancer has been proposed. BRCA2 mRNAs were only detected in dividing cells but not at all in quiescent cells (1-5). Recently, we have found an Alu-repeat containing transcriptional silencer at the upstream of human BRCA2 gene (6). This silencer is active only in the quiescent cells but not in the dividing breast cells, thus explaining the absence of BRCA2 mRNA in the quiescent cells. The mechanisms of the activation and inactivation of this silencer in the quiescent and dividing cells, respectively, are presently unknown. We have shown that specific nuclear proteins from quiescent breast cell nuclear extract sequence-specifically binds to this silencer (6). Understanding the structure-activity relationships in these bindings in reference to covalent modifications of the DNA elements and the protein factors may reveal the mechanisms of the regulation of the silencer function. Thus, we believe that the human BRCA2 gene is silenced in the quiescent stage of breast cells but is activated in the dividing cells by the inactivation of an Alu-containing silencer located at the upstream of the BRCA2 gene promoter. Possible transient epigenetic malfunction in this silencer inactivation process by environmental factors in the dividing cells may lead to defect in DNA repair and subsequence onset of mutations in any key gene leading to oncogenesis. Since there are indications that the development and progression of breast cancer in African Americans may be different from that of Caucasians (7-16), we planned to explore whether the BRCA2 silencer turn-on and turn-off mechanisms are altered in the breast cells isolated from African American females.

Body

Task 1: **To evaluate further the function of BRCA2 gene silencer and the silencer binding protein(s) in human breast epithelial cells of different ethnic origin.**

- **Test various human breast cell lines for their BRCA2 gene silencer (221 bp) activity**: The map of the human BRCA2 gene transcriptional silencer is shown in Fig. 1. We made two constructs (Fig. 2) to test the activity of this silencer in several lines of human breast cells of African American and Caucasian origins (Table 1). The cells were serum-starved to take them to the quiescent stage. In a parallel study, we found that the silencer works only at the quiescent stage of the cells. It is inactive in the dividing cells. With the exception of MDA-MB-157, the cells of African American origin showed less activity of the silencer as compared to the cells of Caucasian origin. Table 1 shows the silencer activities with the SV40 promoter/enhancer system. Similar activities were found with the BRCA2 promoter/enhancer system (not shown). Some of cells of African American origin multiply poorly in the growth medium prescribed by ATCC and we could not store them in liquid nitrogen. Thus, among the cells of African American origin listed in Table 1, we are now working only with MDA-MB-157, Hs 275.T, and MDA-MB-468 cells.

Table 1. BRCA2 gene silencer (221 bp) activity in various human breast cell lines

Cell line Name	ATCC #	Breast Cell Nature	Silencer Activity[a]
African-American origin (Black female)			
Hs 927.T	**CRL-7679**	**Fibrocystic disease***	32 ± 3
MDA-MB-157	HTB-24	Breast carcinoma	69 ± 1
Hs 275.T	CRL-7224	Breast carcinoma	27 ± 3
MDA-MB-468	HTB-132	Adenocarcinoma	13 ± 2
Hs 496.T	CRL-7303	Breast carcinoma	44 ± 6
Hs 467.T	CRL-7809	Breast carcinoma	41 ± 5
Caucasian origin (White female)			
MCF-10F	**CRL-10318**	**Fibrocystic disease***	62 ± 7
MCF-7	HTB-22	Adenocarcinoma	81 ± 4
MDA-MB-231	HTB-26	Adenocarcinoma	72 ± 3
MDA-MB-361	HTB-27	Adenocarcinoma	67 ± 8
MDA-MB-436	HTB-130	Adenocarcinoma	73 ± 1
MDA-MB-453	HTB-131	Breast carcinoma	86 ± 4

*'Normal' breast cells; [a]Silencer activity in the cells transfected with the silencer containing reporter plasmid (Fig. 2) is expressed as % inhibition of the normalized luciferase activity in cells transfected with the control plasmid without the silencer. Values are Mean ± SE (n=6).

- **Isolate nuclear extracts from the cells and test by band-shift assay whether there is any correlation between the silencer activity and the level of silencer binding proteins:** We prepared nuclear extracts from quiescent cultures of the cells listed in Table 1 and performed electrophoretic mobility shift assays using ^{32}P-end-labeled 221 bp silencer fragment as probe. This study indicated good correlation of the silencer activity in the cell with the intensity of the specific DNA/protein bands in the autoradiogram (Fig. 3). Extracts from Hs.275T and MDA-MB-468 cells did not show any detectable shifted band in the autoradiogram under the condition of the assay (Fig. 3).

Task 2: **To identify the sequence elements in the BRCA2 gene silencer responsible for the activity of the silencer by mutational analysis.**

- **Purify partially the silencer binding proteins (BSBPs) from breast cell nuclear extracts:** We purified proteins in the MCF-7 and MDA-MB-231 cell nuclear extracts that binds to the 221 bp silencer sequence. We amplified the 221 bp silencer sequence with one of the primers used tagged with biotin at the 5'-end. The quiescent cells were incubated for 16 h in methionine-free medium with ^{35}S-methionine to radiolabel all the proteins synthesized inside the cell. Nuclear extracts were made and incubated with the biotin tagged silencer . The silencer-protein complex was then captured with streptavidine-paramagnetic particles in a

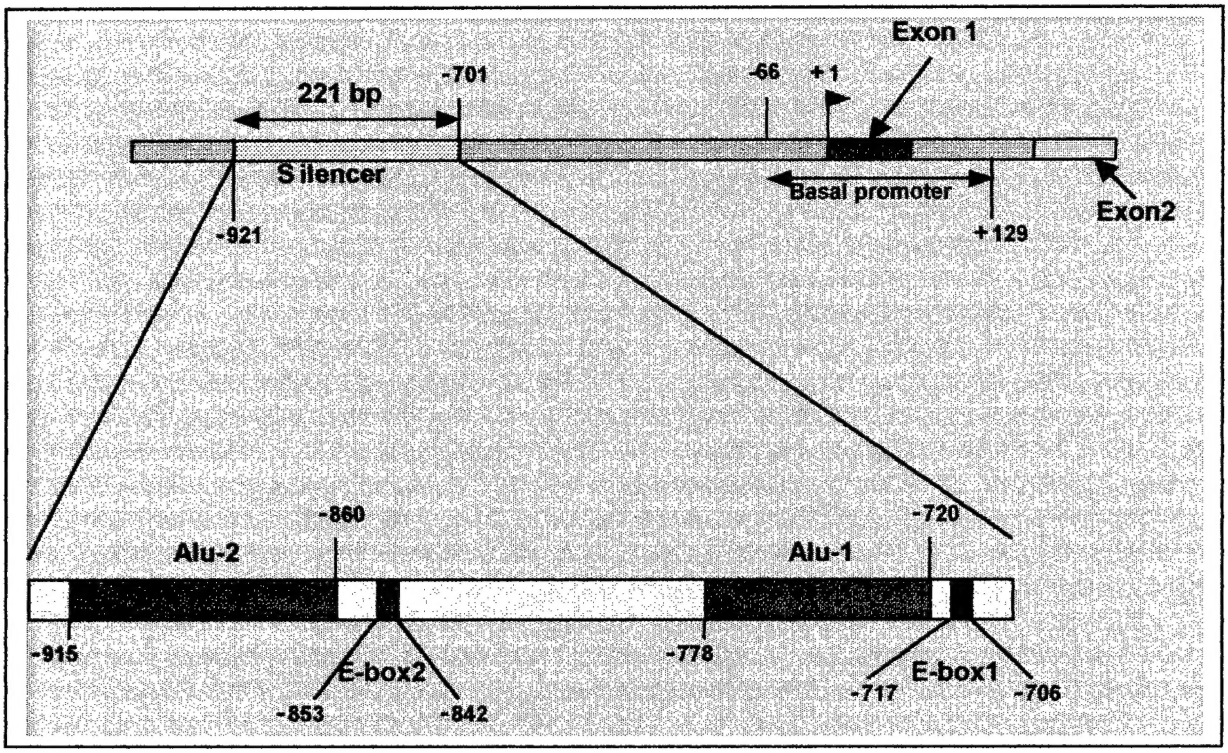

Fig. 1. Sketch showing the relative positions of the human BRCA2 promoter and silencer sequences. The silencer has two Alu-boxes each with a non-canonical E-box sequence.

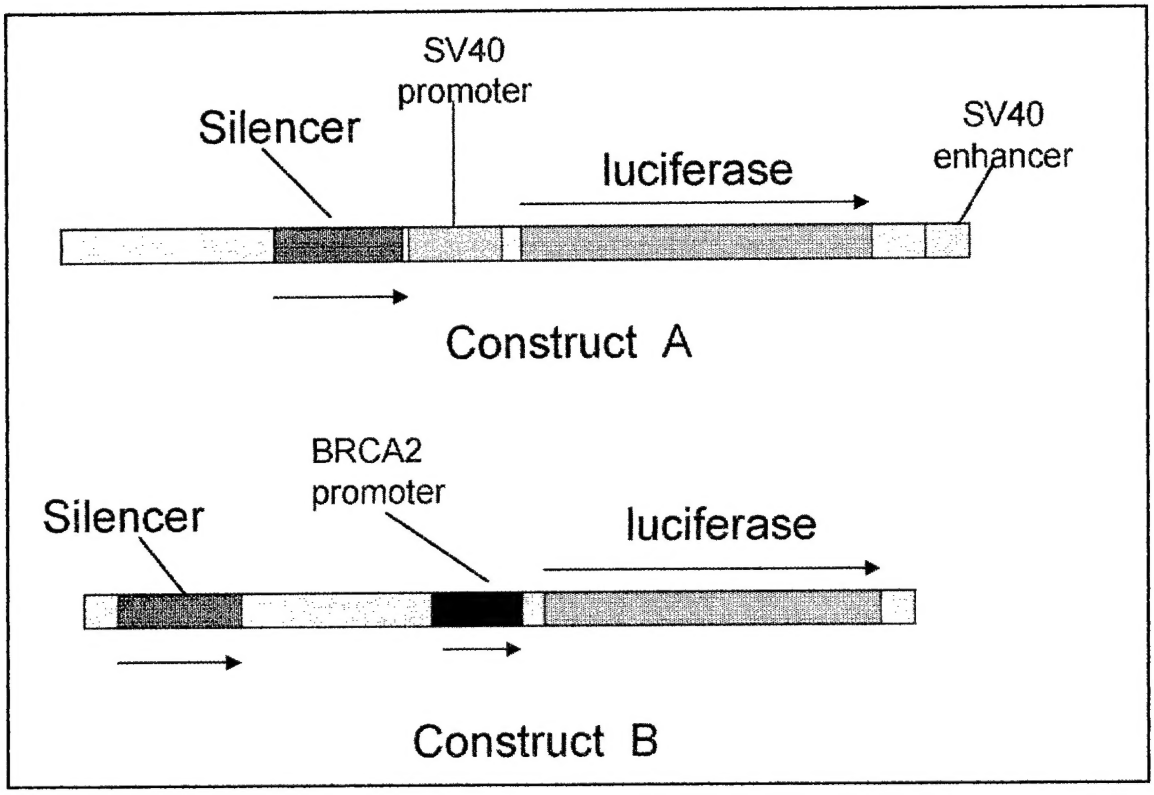

Fig. 2. Test constructs made with the human BRCA2 silencer. Construct A has SV40 promoter/enhancer system whereas construct B has the promoter/enhancer system of human BRCA2 gene.

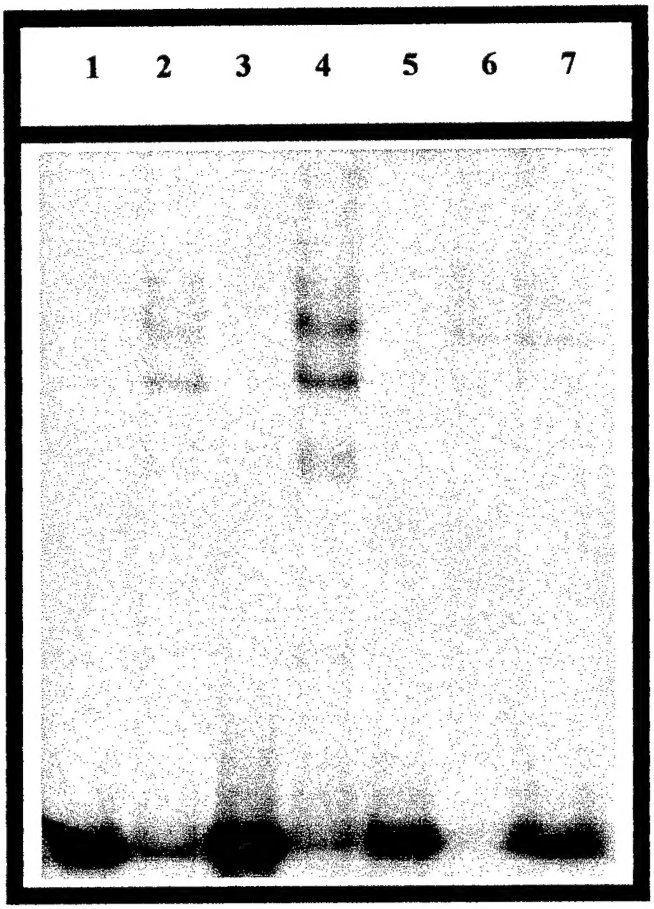

Fig. 3. EMSA analysis for the binding of nuclear proteins from the extracts of several human breast cells. Lane 1, No nuclear extract; Lanes 2-6, nuclear proteins (1 microgm) from MCF-7, MDA-MB-468, MDA-MB-231, Hs275.T, Hs 496.T and Hs 467.T, respectively.

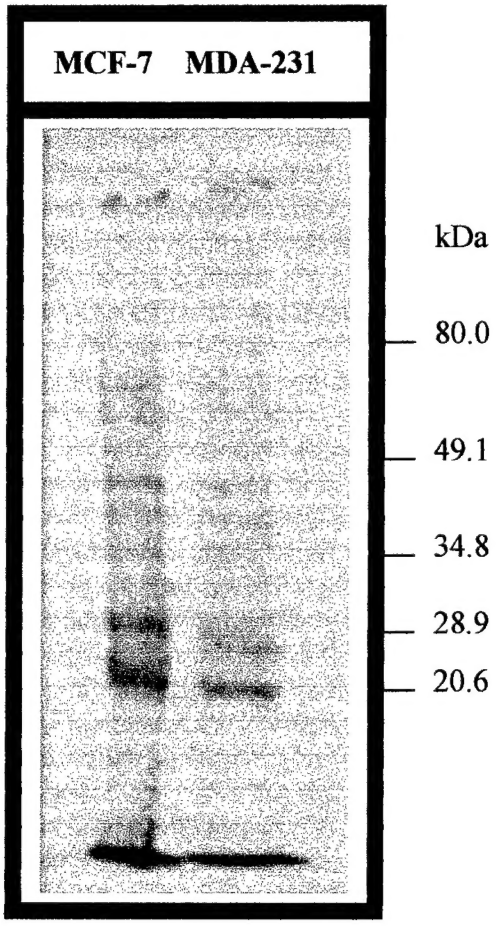

Fig. 4. Autoradiogram showing proteins affinity purified from the nuclear extracts of MCF-7 and MDA-MB-231 cells using biotin-tagged 221 bp human BRCA2 silencer.

magnetic stand, washed twice with ice-cold PBS. The bound proteins were denatured and reduced in Laemmli's solution and were analyzed by SDS-PAGE followed by autoradiography. Six or seven distinct protein bands were visible in the autoradiogram from the two different cell types (Fig. 4).

Key Research Accomplishments

- We tested various human breast cell lines for their BRCA2 gene silencer (221 bp) activity to find reduced activity in many breast cells of African American origin.
- The silencer binding proteins in nuclear extracts from the breast cells seem to vary in relative concentrations depending upon the ethnic origin of the cells.
- We have partially purified the silencer binding proteins (BSBPs) from breast cell nuclear extracts.

Reportable Outcomes

None yet.

Conclusions

The BRCA2 gene silencer seems to be less active in the breast cells of African American origin than in those of Caucasian origin. Whether this effect is also differential in the dividing cells needs to be determined. If the silencer is active in dividing cells, that may cause defect in the DNA repair mechanisms and may induce mutations. The function of the silencer appears to be correlated with the relative concentration of the nuclear proteins that bind to it. We found two major and 4-5 minor proteins that bind to the silencer DNA segment. Foot-print analysis with the purified proteins and mutational analysis of the elements in the silencer will clarify the questions posed by these observations.

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